AMENDMENT

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

In the Claims:

1. (Currently Amended) A ~~M~~method for ~~the determination of~~ determining the identity of at least one nucleotide in a RNA-molecule comprising the steps of:
(a) providing a single stranded form of the RNA-molecule;
(b) hybridizing an oligonucleotide primer ~~binding~~ to a predetermined position of the RNA molecule, whereby the hybridization is performed in the presence of at least one RNAse-inhibiting agent;
(c) performing at least one primer extension reaction in an extension reaction solution, whereby the oligonucleotide primer is extended on the RNA-molecule through incorporation of at least one nucleotide by the action of a RNA dependent polymerase, whereby the polymerase is a reverse transcriptase (RT) that essentially lacks RNase H activity;
(d) detecting the presence or absence of incorporation, thereby indicating the nucleotide identity of the RNA molecule in the relevant position;
Whereby step (c) to (d) optionally are repeated.

2. (Currently Amended) The ~~M~~method according to claim 1, whereby step (c) to (d) are repeated.

3. (Currently Amended) The ~~M~~method according to claim 1 ~~or 2~~, whereby the incorporated nucleotide(s) is (are) recorded.

4. (Currently Amended) The ~~M~~method according to claim 1 ~~3~~, whereby the presence or absence of incorporation is indicated by the presence of a detectable moiety.

5. (Currently Amended) The ~~M~~method according to claim 4, wherein the detectable moiety is removed or neutralized in step (d) after the detection.

6. (Currently Amended) The ~~M~~method according to claim 1~~-5~~, whereby the primer extension reaction results in the release of a residue molecule.

7. (Currently Amended) The ~~M~~method according to claim 6, whereby the primer extension reaction results in the release of a PPi molecule only upon incorporation of a nucleotide.

8. (Currently Amended) The ~~M~~method according to claim 7, wherein ~~step (c) is performed by including~~ the extension reaction solution comprises enzymes, comprising luciferase, apyrase, and ATP-sulfurylase, and reagents to detect light triggered by the release of PPi ~~to trigger the release of light~~.

9. (Currently Amended) The ~~M~~method according to claim 1~~-8~~, whereby at least one nucleotide is labeled, such as fluorescently of radioactively, thereby allowing the detection of step (c) to be performed by means of detecting the presence or absence of a labelled nucleotide.

10. (Currently Amended) The ~~M~~method according to claim 9, whereby the label on the labelled nucleotide is cleavable.

11. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby the detection of step (c) is performed by means of detection of a change in physical properties of the RNA-molecule.

12. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby the RT polymerase is chosen from the group comprising: HIV-1 RT, M-MuLV RT, AMV RT, RAV2 RT, Thermoscript AMV RT, Superscript II M-MuLV RT, Tth DNA polymerase, Superscript II RNase H RT.

13. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby a mixture of RNA dependent polymerases is added to the reaction mixture of step (a).

14. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby the extension reaction is performed at a temperature ranging from 28 to 70 °C.

15. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby ~~the pH of~~ the extension reaction solution has a pH ~~is~~ in the interval from 7.6 to 8.6~~, preferably from 8.0 to 8.4~~.

16. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 8, whereby the extension reaction solution further comprises a concentration of deoxynucleotides ~~is~~ in ~~the~~ an interval from 1 μM to 1 mM.

17. (Currently Amended) The ~~M~~mMethod according to ~~any one of the preceding~~ claims 8, whereby the extension reaction solution further comprises a salt concentration ~~of the reaction mixture is~~ in ~~the~~ an interval from 10~~0~~ to 100 mM.

18. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, wherein the oligonucleotide primer is a DNA primer.

19. (Currently Amended) The ~~M~~method according to claim 18, whereby the ~~nucleotide is the deoxynucleotide~~ DNA primer comprises dATP, which ~~further~~ is exchanged for ~~the analogue~~ alpha-S-dATP.

20. (Currently Amended) The ~~M~~method according to claim 1~~-18~~, wherein the oligonucleotide primer is a RNA primer.

21. (Currently Amended) The ~~M~~method according to claim 20, whereby the RNA primer comprises ~~nucleotide~~ ATP, which is exchanged for ~~the analogue~~ alpha-S-ATP.

22. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 8, whereby the extension reaction solution further comprises a RNA-secondary structure reducing reagent~~, preferably chosen~~ selected from the group ~~comprising~~ consisting of T4 Gene 32 Protein, retroviral nucleocapsid protein, actinomycin D, glycerol, methyl mercury hydroxide, methoxyamine-bisulfite, DMSO, spermidine, formamide, SSB (single stranded binding protein) and blocking primer~~, is included in the extension reaction~~.

23. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby the RNA molecule is subjected to an RNA amplification prior to the extension reaction.

24. (Currently Amended) The ~~M~~method according to claim 23, whereby the RNA amplification comprises exchanging rITP for rGTP~~the nucleotide rITP is exchanged for rGTP in the amplification~~.

25. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, wherein the oligonucleotide primer is immobilised to a solid phase or wherein the RNA molecule is captured to a solid phase by an immobilized oligonucleotide.

26. (Currently Amended) The ~~M~~method according to ~~any one of the preceding~~ claims 1, whereby ~~the~~ a quantity of the RNA-molecule is determined by measuring the intensity of the incorporation signal and comparing it to a reference.

27. (Currently Amended) A ~~K~~kit for performing the nucleotide identification of claim 1~~-26~~, comprising in separate vials a reverse transcriptase that essentially lacks RNase H activity, nucleotides, necessary enzymes for a sequencing-by-synthesis reaction, and optionally other necessary reagents.

28. (Currently Amended) The ~~K~~kit according to claim 27, which further comprises a RNA quantity reference sample.

29. (Currently Amended) A ~~M~~method for determining the sequence of a ribonucleic acid molecule comprising the steps of;

a) providing a single-stranded form of said ribonucleic acid molecule;

b) hybridizing a primer to said single stranded form of said ribonucleic acid molecule to form a template/primer complex, whereby the hybridisation is performed in the presence of at least one RNase-inhibiting agent;

c) enzymatically extending the primer by the addition of an RNA dependent polymerase and a mixture of nucleotides and a derivative of said nucleotides, wherein the derivative of said nucleotide comprises a label linked to a nucleotide via an optionally cleavable link and wherein the proportion in the mixture between the nucleotides and the derivative of said nucleotide is within the range of 1-60%, 1-50%, 1-40%, 1-30%, or 1-20%, ~~preferably in the range of 5-60%, 5-50%, 5-40%, 5-30%, or 5-20%, or more preferably in the range of 10-60%, 10-50%, 10-40%, 10-30%, or 10-20%,~~ whereby the polymerase is a reverse transcriptase that essentially lacks RNase H activity; and

d) determining the type of nucleotide added to the primer.

30. (Currently Amended) The ~~M~~method according to claim 29, wherein the label is neutralized after step d) by the addition of a label-interacting agent or by bleaching~~, preferably by photo-bleaching~~.

31. (Withdrawn) Kit comprising, in separate compartments, a mixture of natural nucleotides and a derivative of said nucleotides according to step c) of claim 29, and at least one of the following components: a reverse transcriptase that essentially lacks RNase H activity, a reducing agent, a carrier, a capping agent, an apyrase, an alkaline phosphatase, a PP-ase, a single strand binding protein or the protein of Gene 32, for performing the method according to claim 29-30.

32. (Withdrawn) Kit according to claim 31, which further comprises a RNA quantity reference sample.

33. (Withdrawn) Method for determining the sequence of a ribonucleic acid molecule comprising the steps of:

a) providing a single-stranded form of said ribonucleic acid molecule;

b) hybridizing a primer to said single stranded form of said ribonucleic acid molecule to form a template/primer complex;

c) enzymatically extending the primer by the addition of an RNA dependent polymerase and a mixture of nucleotides and a derivative of said nucleotides, wherein the derivative of said nucleotide comprises a label linked to a nucleotide via an optionally cleavable link and wherein the proportion in the mixture between the nucleotides and the derivative of said nucleotide is within the range of 1-60%, 1-50%, 1-40%, 1-30%, or 1-20%, preferably in the range of 5-60%, 5-50%, 5-40%, 5-30%, or 5-20% or more preferably in the range of 10-60%, 10-50%, 10-40%, 10-30%, or 10-20%.

d) determining the type of nucleotide added to the primer;

34. (Withdrawn) Method according to claim 33, wherein the label is neutralized after step d) by the addition of a label-interacting agent or by bleaching, preferably by photo-bleaching.

35. (Withdrawn) Kit comprising, in separate compartments, a mixture of natural nucleotides and a derivative of said nucleotides according to step c) of claim 33, and at least one of the following components; an RNA dependent polymerase, a reducing agent, a carrier, a capping agent, an apyrase, an alkaline phosphatase, a PP-ase, a single strand binding protein or the protein of Gene 32, for performing the method according to claim 33-34.